Effects of Microplastic Particles and Microplastic Leachate on the Germination and Growth of *Lolium multiflorum*

Sofia Vikfors
University of Helsinki
Faculty of Biological and Environmental Sciences
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HELSINGIN YLIOPISTO
HELSINGFORS UNIVERSITET
UNIVERSITY OF HELSINKI

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Tiivistelmä – Referat – Abstract

Microplastics (MPs) are widespread environmental pollutants that have been detected in virtually all environmental compartments. Despite this, research has mainly focused on the impacts of microplastic on shorelines and at sea. The effects of MPs on terrestrial ecosystems has been sparsely investigated, and there are only a few studies on direct effects on terrestrial plants. Although plastic polymers are considered inert and non-hazardous, toxic additives are often added to the polymers during manufacture which may leach out into the environment, displaying ecotoxic effects. In this work, the effects of microplastic particles and microplastic leachate on the germination and growth of *Lolium multiflorum* (Italian ryegrass) was investigated. High density polyethylene (HDPE), which is one of the plastic polymers with the largest annual production, was chosen as the plastic material for investigation. New MPs, artificially aged MPs, MPs from the Lahti region, and MPs from Port Elizabet, South Africa was used in parallel to compare the effects of ageing and regional environmental factors on the ecotoxicity of MPs. The total germination percentage, mean germination rate, synchronization index, germination index, and time to 50% germination was investigated, as well as the root lengths, shoot lengths, root/shoot ratio, and fresh weights of the seedlings.

The results showed that exposure to new and Lahti MPs and leachates severely inhibited the extent and speed of the germination of *L. multiflorum*, whereas all categories of MPs and leachates inhibited the growth to some extent. Most severe inhibition in germination and growth was seen for the new MP and new leachate, followed by Lahti MP and Lahti leachate. The root growth, shoot growth, and plant biomass were also severely reduced for these exposure media. For the aged and Port Elizabeth material, there were slight but significant ($p < 0.05$) inhibition in root lengths and fresh weights, but no significant inhibition in the germination parameters. These findings indicate that ageing severely reduces the ecotoxic effects of MPs, and that regional environmental factors affect the ecotoxicity of MPs. Microplastics from Port Elizabeth were significantly less toxic to *L. multiflorum* than MPs from the Lahti region, possibly due to the warmer climate in South Africa. Another explanation could be that the plastic material collected in Port Elizabeth wast older than the one from Lahti. There was little to no difference in germination and growth between seeds exposed to MPs or leachates of the same origin, indicating that it is the substances leaching out of the MPs that are responsible for their ecotoxicity.

Avainsanat – Nyckelord – Keywords
Microplastics; leachate; HDPE; germination; growth; germination rate; synchronization index; germination index; T_{50}

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Tiivistelmä – Referat – Abstract

Mikroplast (MP) är en utbredd miljöförorening som har upptäckts i praktiskt taget alla avdelningar av miljön. Trots detta har forskning huvudsakligen fokuserat på effekterna av mikroplast på strandlinjer och till havs. Effekterna av mikroplaster på land har studerats sparsamt och det finns bara några få studier som direkt undersöker deras effekter på landväxter. Även om plastpolymerer anses vara inerta och icke-toxiska, adderas ofta giftiga tillsatser till polymererna under tillverkningen vilka kan läcka ut miljön och uppvisa ekotoxiska effekter. I detta arbete undersöktes effekterna av mikroplastpartiklar och mikroplasteluenter på grobarheten och tillväxten av *Lolium multiflorum* (italienskt rajgräs). Högdensitetspolyeten (HDPE), som är en av plastpolymererna med den största årliga produktionen, valdes som plastmaterial för undersökning. Ny MP, artificiellt åldrad MP, MP från Lahti-regionen och MP från Port Elizabeth, Sydafrika användes parallellt för att jämföra effekterna av åldrande och regionala miljöfaktorer på mikroplasternas ekotoxicitet. Total groningsprocent, genomsnittlig groningshastighet, synkroniseringsindex, groningsindex och tid till 50% groning undersöktes, såväl som plantornas rotlängd, skottlängd, rot/skottförhållande och färsk vikt.

Resultaten visade att exponering för mikroplastpartiklar och mikroplasteluenter av två ursprung; ny och Lahti, kraftigt hämmade omfattningen och hastigheten av *L. multiflorums* grobarhet, medan alla kategorier av partiklar och eluenter hämmade dess tillväxt i viss utsträckning. Den allvarligaste hämningen av grobarhet och tillväxt sågs för ny MP och nya eluenter, följt av partiklar och eluenter från Lahti. Rottillväxten, skottillväxten och växtbiomassan reducerades också kraftigt för dessa exponeringsmedier. För det åldrade och Port Elizabeth-materialet sågs en liten men signifikant ($p < 0,05$) hämning i rotlängd och färsk vikt, men ingen signifikant hämning i grobarhets-parametrarna. Dessa resultat tyder på att åldrande kraftigt minskar mikroplastens ekotoxiska effekter och att regionala miljöfaktorer påverkar mikroplastens ekotoxicitet. MP från Port Elizabeth var signifikant mindre giftiga för *L. multiflorum* än MP från Lahti-regionen, möjligen på grund av det varmare klimatet i Sydafrika. En annan förklaring kan vara att det plastmaterial som samlats in i Port Elizabeth var äldre än det från Lahti. Det fanns liten till ingen skillnad i grobarhet och tillväxt mellan plantor utsatta för partiklar eller eluenter av samma ursprung, vilket tyder på att det är ämnena som läcker ut från mikroplasten som är ansvariga för deras ekotoxicitet.

Avainsanat – Nyckelord – Keywords
Mikroplast; eluenter; HDPE; grobarhet; tillväxt; groningshastighet; synkroniseringsindex; groningsindex; T_{50}

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1 Introduction

The global plastic production increased from 2 million tons in 1950 (Geyer *et al.*, 2017) to 359 million tons in 2018 (Plastics Europe, 2019). In this relatively short period of time, approximately 5 billion tons of plastic has ended up in landfills or the natural environment (Geyer *et al*., 2017). The estimated half-lives of some plastics can be up to several thousands of years (Chamas *et al*., 2020), which makes the accumulation of plastic waste in the environment a matter of ever-increasing concern. When plastic pollution was first recognized, research focused on larger plastic debris (Duis & Coors, 2018). However, in the last fifteen years or so, most of the public and scientific attention has been directed towards microplastics (MPs). MPs are commonly described as plastic particles smaller than 5 millimeters (Betts, 2008) and enter nature in both primary and secondary forms. Primary MPs are produced as such and used e.g. as pre-production resin pellets, in industrial abrasives or in personal care products, whereas secondary MPs are formed when larger pieces of plastic break down (Rillig, 2012; Koelmans *et al*., 2014). This takes place in nature due to mechanical weathering, UV-radiation and possibly by earthworm digestion, but secondary MPs are also formed, for example, when synthetic clothes are washed (Rillig, 2012).

MPs in the environment may be ingested by organisms and thereby pose a direct threat to them, in addition to acting as vectors for transporting hydrophobic pollutants that sorb to the surface of the plastic material (Koelmans *et al*., 2014). These pollutants either originate from the surrounding environment or are already present in the MPs when they arrive in nature. Plastic polymers themselves are considered inert and non-hazardous, although the monomers they are made up of may be toxic. An example of this is polycarbonate (PC), which consist of the known endocrine disruptor bisphenol A (Lithner *et al*., 2011). Inherently toxic or not, hazardous additives are often added to

the plastics during manufacture to give them desired properties (Lithner *et al*, 2011; Koelmans *et al*., 2014). There are several thousands of plastic additives in use, including various stabilizers, flame retardants, pigments and fillers (Lithner *et al.*, 2011). This gives rise to a complex chemical cocktail, whose risk of being released into the environment increases as plastic accumulates in nature. The effects of such chemical mixtures on biota is largely unknown.

Although MPs have been detected in virtually all environmental compartments, including oceans, sediments, soil, freshwater, air, and biota (Thompson *et al*., 2004; Zhang & Liu, 2018; Biginagwa *et al*., 2015; Dris *et al*., 2016; Lusher *et al.*, 2013), the vast majority of research has focused on the impacts of microplastic on shorelines and at sea (Barnes *et al*., 2009). Only comparably recently, the possibility that microplastic may pose a threat also to terrestrial organisms has received attention (Rillig, 2012). Studies on direct effects of microplastics and microplastic leachates on organisms are sparse, and there is almost a complete lack of research in the context of terrestrial plants. The purpose of this study was to increase our knowledge on this novel subject by examining the effects of MP particles and MP leachate on the terrestrial plant *Lolium multiflorum*. This short-lived perennial grass, which is also called Italian ryegrass, is widespread in the environment and an important species for forage production in temporary grassland (Peter-Schmid *et al.*, 2010).

High-density polyethylene (HDPE) was chosen as the plastic material for this study, as it is one of the plastic polymers that has the largest annual production (Lithner *et al*., 2011; Plastics Europe, 2019) and is a major contributor to the MP pollution of soil (Kawecki & Nowack, 2019). HDPE is globally produced in approximately 32 million tons every year, exceeded only by polypropylene (PP) (45 million tons) low-density (LD) and linear-low-density (LLD) PE (39 million tons), polyvinylchloride (PVC) (37

million tons), and polyethylene terephthalate (PET) (33 million tons) (Lithner *et al*., 2011). Typical applications of HDPE include bottles, toys, pipes, food packaging, car fuel tanks, wire coatings, and cable insulations (Vasile & Pascu, 2005). HDPE is made up of the monomer ethylene, which is a colorless gas that may cause irritation to eyes, skin, and the respiratory tract upon exposure (Lithner *et al*., 2011). Although HDPE is considered one of the least hazardous of the plastic polymers (Lithner *et al*., 2011) plastic additives leaching out of this material has been found to cause acute toxicity to *Daphnia magna* (Lithner *et al*., 2009) and to have estrogenic activity (Yang *et al*., 2011). Polyethylene leachate has also been demonstrated to significantly impair the growth and photosynthetic capacity of the photosynthetic bacteria *Prochlorococcus* (Tetu *et al*., 2019), and the development of larvae of the sea-urchin *L. variegatus* (Nobre *et al.*, 2015).

This study investigated the germination and growth of *L. multiflorum* under exposure to HDPE MPs and HDPE MP leachates, since these are the plant responses that most accurately indicate changes to environmental quality (Gvozdenak *et al*., 2011). Both new and artificially aged plastic were used, together with plastic collected in the Lahti region and from a beach in Port Elizabeth, South Africa. The different categories were chosen to examine whether ageing or regional environmental factors affect the ecotoxicity of microplastics. MP particles as well as leachates from the MP materials were used in parallel, to identify possible differences in ecotoxicity between the particles themselves and their corresponding leachates. The experiments were conducted under laboratory conditions, with the concentrations of MP particles and MP leachates exceeding those typically found in nature. However, these conditions can be assumed to represent point sources of plastic contamination, or the accumulating amount of plastic in the terrestrial ecosystems towards which we are heading. The

hypotheses of this study was that exposure to MP particles and MP leachates have negative impacts on the germination and growth of *L. multiflorum*, and that differences in age and origin of the microplastic affects the impairment to varying extent.

2 Materials and Methods

2.1 Microplastics, Aged Microplastics and Plastic Leachate

Commercial soda bottle caps of high-density polyethylene (HDPE) were used as the plastic material for the experiments. To obtain the microplastic material, the bottle caps were fragmented into MP particles of an average size of 4 mm ± 1 mm with a SHR3D IT compact plastic shredder (3devo B.V. Utrecht, Netherlands). Four different categories of bottle caps were used in parallel; new bottle caps, artificially aged bottle caps, bottle caps collected in the Lahti region, and bottle caps collected on a beach in Porth Elizabeth, South Africa. The collection, artificial ageing, and leaching of the bottle caps had been carried out prior to the experiments conducted for this thesis.

Accelerated aging of the MP material was accomplished according to Fejdyś *et al*. (2011) using PN-EN 12280-1:2002. Before this, the plastic material was washed with ISO reconstituted water (pH 7.2) (International Organization for Standardization, 1996) and dried at 25 °C to remove any superfine particles. The accelerated aging was carried out in a thermal chamber (TK 720 Binder GmbH, Tuttlingen, Germany) with a heating effect at a temperature of 70 °C ± 0.5 °C and humidity of 0 ± 1.5 % for 160 days.

Treatment to induce leaching of the MP was done according to the Swedish standard 12457: 2003 (Swedish Standard Institute, 2003), with some modifications. Leaching time was increased from 24 h to 72 h and a temperature of 50 °C was used. These modifications were applied to enhance the leaching and to simulate a more realistic scenario. When exposed to sunlight outdoors, the surface of plastic material in the

environment may reach temperatures even beyond 50 °C (Wypych, 1999). To accomplish the leaching, 200 g of MP material was mixed with ISO reconstituted water (pH 7.2) (International Organization for Standardization, 1996) in a liquid-to-solid ratio (L/S) of 10. The whole process was done in the dark, in round bottom flasks on a rotary mixer (Hei-VAP Valve, Heidolph-Instruments, Schwabach, Germany) at 21 rpm. After leaching, the liquid was separated from the solids by vacuum filtration. Whatman borosilicate glass microfiber filter (grade GF/F; particle retention 0.7 µm), which are approved in the EPA method TCLP 1311 (United States Environmental Protection Agency, 1992) for toxicity characteristic leaching procedure, were used for the filtration.

2.2 *L. multiflorum* Seeds

Commercially available seeds of *L. multiflorum* were used for the experiments. The seeds were soaked in tap water for 24 h before sowing to induce germination. After soaking, whole and undamaged seeds were selected and planted individually in 16 x 100 mm soda glass test tubes, containing different exposure media.

2.3 Exposure Media

Nine categories of exposure media were used in parallel; untreated controls, soil containing 3% w/w of microplastic particles of new bottle caps (from now on called new MP), soil watered with 4 mL leachate of new bottle caps MP (new leachate), soil containing 3% w/w of microplastic particles of aged bottle caps (aged MP), soil watered with 4 mL leachate of aged bottle caps MP (aged leachate), soil containing 3% w/w of microplastic particles of bottle caps from Lahti (Lahti MP), soil watered with 4 mL leachate of bottle caps MP from Lahti (Lahti leachate), soil containing 3% w/w of microplastic particles of bottle caps from Port Elizabeth (PE MP), and soil watered with

4 mL leachate of bottle caps MP from Porth Elizabeth (PE leachate). The control media consisted of pure garden soil, watered with 4 mL of tap water. For the exposure media containing MP particles, garden soil was mixed with 3% w/w of the MP particles and watered with tap water. The exposure media containing MP leachate contained pure garden soil and was watered with the leachates instead of tap water.

2.4 Experimental Setup

For each exposure media, a total of 20 seeds were planted individually in test tubes arranged in 4 rows á 5 seeds each as seen in Figure 1.

Figure 1. The experimental setup with nine exposure media and 4 x 5 seeds per group. The picture shows only one of six replicates of the experiment, beacause the other replicates were conducted in parallel by different people.

All test tubes contained 9 g of soil and was watered with 4 mL of liquid. The liquid was added to the test tubes before planting to avoid drying of the seeds. The seeds were let

to germinate for seven days in room temperature. To increase reliability, the same experiment was repeated six times by different people; Sofia, Jenohin, Amalia, Hannah, Paul, and Kuysang. Hence, there was a total of 120 seeds planted per exposure media. The other five replicates of the experiment were conducted separately from this thesis, but the data obtained by all six people was combined to one data set before performing the statistical analysis and calculating the results.

2.5 Measured Parameters

The germination and growth of *L. multiflourm* was recorded, because changes to these parameters are good indicators of changes in environmental conditions (Gvozdenak *et al.*, 2011). The amount of germinated seeds for each exposure media was counted every day for seven days after planting. McNair *et al.* (2012) define germination as the "*physiological and developmental processes that result in mature, non-dormant seeds upon exposure to appropriate conditions of water availability, temperature and other physicochemical factors*". In germination experiments, however, it is in fact the *completion* of germination that is observed (McNair *et al.*, 2012). On day 7, one seedling at a time was carefully removed from the test tube and gently washed. The root and shoot length were measured with a digital caliper, and the fresh weight of the seedlings was measured with a digital scale immediately after this to prevent dehydration.

Selected germination parameters were calculated using a data sheet developed by Dr. Farhan Khalid (Khalid, 2016). These included total germination percentage (G %age), mean germination rate (MGR), Synchronization index (Z), germination index (GI), and time to 50% germination (T_{50}). Several methods and mathematical expression that measure the germination process have been developed over the years (Bewley *et al.*,

2013; Soltani *et al*., 2015; Al-mudaris, 1998; Al-Ansari & Ksiksi, 2016). Unfortunately, they sometimes share the same application name although measuring different characteristics or have different symbols or mathematical expressions while measuring the same aspect of the germination process (Ranal & Santana, 2006). Explanations of the parameters used in Khalid's data sheet for measuring the germination process is described in the following sections.

Total G %age

The total G %age measures the proportion of seeds that germinate under a given period of time; in this case 7 days. Thus, the higher the G %age value, the greater the germination of a seed population (Al-mudaris, 1998). The total G %age was calculated according to the following formula by Dastanpoor *et al.*, 2013:

$$G\ \%age = \frac{n}{N} \times 100$$

where *n* is the number of germinated seeds and *N* is the total number of seeds in the population. In this experiment, *n* represents the cumulative number of germinated seeds, counted every day for 7 days after planting.

MGR

The mean germination rate is the inverse of the mean germination time (MGT), which is defined as the time at which 50% of all germinating seeds have completed germination (Bewley *et al*., 2013, Soltani *et al*., 2015). Lower values of MGT indicate earlier and more uniform germination (Dastanpoor *et al.*, 2013), thus, lower values of MGR is associated with more offset and spread germination.

MGT is calculated as described by Bewely *et al.* (2013):

$$MGT = \frac{\sum(t \cdot n)}{\sum n}$$

where *t* is the time in days, starting from day 0 (the day of sowing) and *n* is the number of seeds germinating on day *t*. Consequently, MGR was calculated as:

$$MGR = \frac{\sum n}{\sum(t \cdot n)}.$$

Z

The synchronization index (Z) measures the degree of overlap in germination of two seeds in the same replication of a treatment (Ranal & Santana, 2006). Z can assume values between 0 and 1, where Z = 1 describes a case where all seeds germinate at the same time, and Z = 0 represent at least two seeds germinating at different times (Ranal & Santana, 2006). If no pair of seeds finish germination at the same time, the Z does not produce a number. A problem that can arise with synchrony, is that short intervals between observations yield a low value for Z, because few seeds are counted at the same time. If the germination of each seed is detected individually, there would appear to be no synchronicity, and oppositely, if the interval between consecutive measurements is too high, the synchrony may be overestimated. (Ranal & Santana, 2006). The synchronization index (Z) was calculated according to Ranal and Santa (2006):

$$Z = \frac{C_{n_i,2}}{N}$$

where $C_{n_i,2} = n_i, (n_i - 1)/2$ and $N = \sum n_i(\sum n_i - 1/2)$. $C_{n_i,2}$ is a combination of the seeds germinated in the time i, two together, and n_i is the number of seeds germinated in the time i.

GI

The germination index (GI) is a measure of both quantity and rate of germination. A higher GI indicates a higher and faster germination. There are several different formulas for calculating GI that measure slightly different things (Al-mudaris, 1998; Al-Ansari & Ksiksi, 2016; Association of Official Seed Analysis, 1983). However, the one used in this test was the one described by the Association of Official Seed Analysis (1983), calculates as:

$$GI = \frac{No.of\ germianted\ seeds}{Days\ of\ first\ count} + \cdots + \frac{No.of\ germinated\ seeds}{Days\ of\ final\ count}.$$

Accordingly, the number of germinated seeds on each individual day after planting was used for the calculation of GI.

T_{50}

The T_{50} describes either time to 50% germination of the final germination percentage within a given period, or time to 50% germination of the total seed population (Bewley *et al*., 2013). If the whole seed population is used for the calculations, the T_{50} would be infinite in cases where the germination is less than 50% (Bewley *et al*., 2013, Soltani *et al*., 2015). Under such circumstances it is recommended use time to another percentile of germination (e.g. time to 20% germination, T_{20}) (Bewley *et al*., 2013, Soltani *et al*., 2015). In the data sheet used for calculating the germination measurements in this experiment, the formula used for T_{50} (by Coolbear *et al*., 1984, developed by Farooq *et al*., 2005) utilizes the time to 50% germination of the total germination percentage. It is worth noting that when using this formula, the T_{50} is based on different percentages of the total population (Bewely *et al*., 2013). If two populations reach 100% germination, they are both compared at 50% germination of the total population.

However, if one population reach 100% germination and one reach 80%, the T_{50} is defined at 50% of the total seed population for the first one, and at 40% for the second one.

T_{50} was calculated according to the formula by Coolbear *et al*., 1984, developed by Farooq *et al*., 2005:

$$T_{50} = t_i + \frac{\left(\frac{N}{2} + n_i\right) \times (t_j - t_i)}{(n_j - n_i)}$$

where N is the final number of germination and n_i, n_j cumulative numbers of seeds germinated by adjacent counts at times t_i and t_j, respectively, when $n_i < N/2 < n_j$.

R/S Ratio

The root/shoot ratio (R/S ratio) of a plant is the relation between the amount of tissues that have supportive functions versus the amount of those that have growth functions (Allaby, 2019). A higher proportion of roots indicate a more effective uptake of soil nutrients, whereas higher fractions of shoots are associated with more light energy uptake (Allaby, 2019). Stress is known to induce changes to the root/shoot ratio in plants, but there is no consistent evidence on whether the root/shoot ratio increase, decrease, or remain unaffected under certain stress conditions (Agathokleous *et al*., 2019). The R/S ratio was calculated for every seedling by dividing the root length with the shoot length. Non-germinated seeds were excluded from the calculations since these would have required division by zero.

2.6 Statistical Analysis

The means and standard deviations were calculated for the total G %age, MGR, Z, GI, T_{50}, root and shoot lengths, R/S ratio, and fresh weights. For the growth parameters,

non-germinated seeds were marked as having root and shoot lengths of 0 mm, and fresh weight of 0 g. This partly explains the large standard deviation in groups with fewer germinated seeds. The standard deviations were adjusted so that the lowest values did not go below 0, as negative root and shoot lengths and weights are impossible.

Analysis of variance (ANOVA) on the germination and growth parameters was performed using the software Rstudio version 1.3.959 (Rstudio Team, 2020) and associated packages cars, onewaytests, lindia, and multcomp. The homogeneity of treatment variance and normality of data distribution were tested using Levene's test and a histogram of standardized residuals plot, respectively. Normal one-way ANOVA was applied for data with homogenous variances and normally distributed residual variation, followed by Tukey's HSD (Honest Significant Differences) pairwise-comparisons to determine the significantly different means ($p < 0.05$). In cases where the equality of treatment variance was not met, Kruskal-Wallis H test with pairwise comparisons was applied instead. Kruskal-Wallis is a non-parametric equivalent to normal one-way ANOVA, in which the observations are ranked, and the rank of each observation is used instead of the actual value in a comparison of the groups' means (Kruskal & Wallis, 1954). The significance values obtained by Kruskal-Wallis pairwise comparisons were adjusted with the Bonferroni correction ($p < 0.05$). Rstudio and the associated package ggplot2 was also used for drawing the graphs in this thesis.

3 Results and Discussion

3.1 Germination

The cumulative percentage of germinated seeds per day, expressed in means ± standard deviation, is presented in Figure 2.

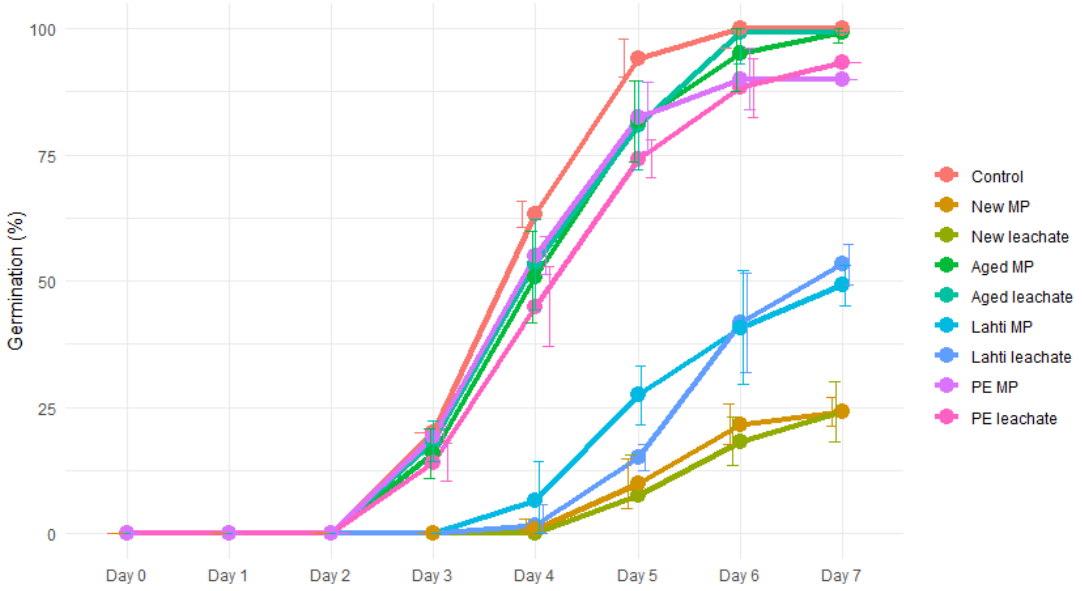

Figure 2. The cumulative germination percentage for different exposure media over a period of 7 days, expressed in means ± standard deviation.

The first seeds germinated three days after sowing in the control group and the groups aged MP, aged leachate, PE MP, and PE leachate. Germination started on day 4 for new MP, Lahti MP, and Lahti leachate, whereas the first seeds from the group new leachate did not germinate until day 5. The germination peak, i.e. the time when the largest amount of seeds germinated, occurred on day 4 for controls, aged MP, aged leachate, PE MP, and PE leachate, on day 5 for Lahti MP, and on day 6 for new MP, new leachate, and Lahti leachate. On day 7, no seeds germinated anymore in the groups control, aged leachate, or PE MP, but a significant amount still germinated in the groups Lahti MP and Lahti leachate. For the remaining groups, a slight germination was still observed on day 7. The results from the pre-ANOVA statistical analysis is presented in Table 1 and Figure 3. The calculated germination parameters expressed in mean values ± standard deviations are presented in Table 2.

Table 1. The results from Levene's test of homogeneity of variance, non-parametric Kruskal-Wallis H test with pairwise comparison, and one-way analysis of variance (ANOVA) test for the final germination percentage (G%age), mean germination rate (MGR), synchronization index (Z), germination index (I), and germination rate of 50% of total seed population (T_{50})

	Levene	Kruskal-Wallis	One-way ANOVA
Variable	F(8, 45)	H(8)	F(8, 45)
G %age	5.256 ***	47.85 ***	
MGR	1.494		45.41 ***
Z	4.224 ***	20.68 **	
GI	1.110		238.1 ***
T_{50}	3.113 **	42.97	

* p < 0.05, ** p < 0.01, *** p < 0.001

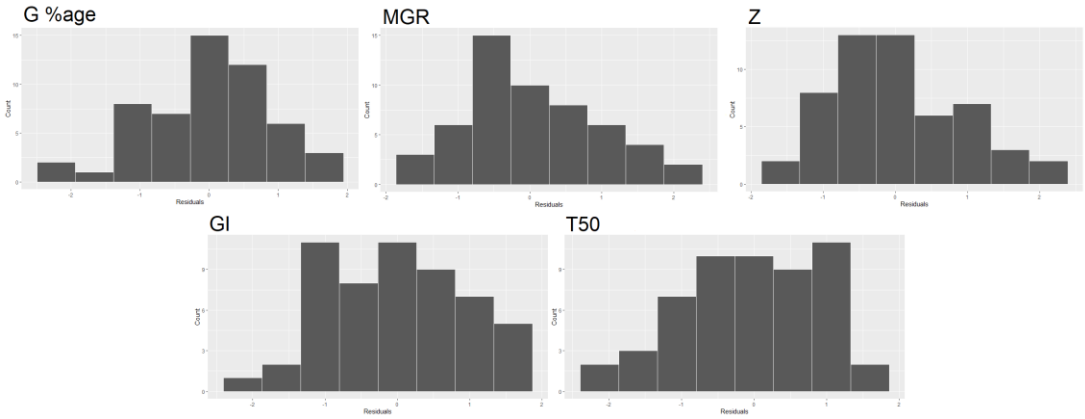

Figure 3. Histogram plots of residuals showing the normality of variance for the final germination percentage (G %age), mean germination rate (MGR), synchronization index (Z), germination index (GI), and germination rate of 50% of total seed population (T_{50}).

Table 2. The total germination percentage (G %age), mean germination rate (MGR), synchronization index (Z), germination index (GI), and time to 50% germination (T_{50}) for the different treatments, expressed in means ± standard deviation. Values not sharing the same letters in the same column differ significantly at the p < 0.05 level.

Treatment	G %age (%)	MGR (day^{-1})	Z (unit less)	GI (day)	T_{50} (day)
Control	100 ± 0.0 ^{a}	0.237 ± 0.004 ^{ab}	0.294 ± 0.024 ^{ab}	4.89 ± 0.06 ^{a}	3.69 ± 0.05 ^{a}
New MP	24.2 ± 5.8 ^{b}	0.177 ± 0.009 ^{c}	0.294 ± 0.077 ^{ab}	0.87 ± 0.22 ^{c}	5.19 ± 0.29 ^{b}
New leachate	24.2 ± 8.6 ^{b}	0.168 ± 0.013 ^{c}	0.389 ± 0.150 ^{a}	0.86 ± 0.41 ^{c}	5.45 ± 0.56 ^{b}
Aged MP	99.2 ± 2.0 ^{a}	0.221 ± 0.014 ^{ab}	0.249 ± 0.037 ^{b}	4.64 ± 0.29 ^{ab}	4.00 ± 0.33 ^{a}
Aged leachate	99.2 ± 2.0 ^{a}	0.224 ± 0.010 ^{ab}	0.249 ± 0.026 ^{b}	4.72 ± 0.22 ^{ab}	3.93 ± 0.29 ^{a}
Lahti MP	49.2 ± 3.8 ^{c}	0.183 ± 0.014 ^{c}	0.308 ± 0.021 ^{ab}	1.85 ± 0.25 ^{d}	4.89 ± 0.47 ^{b}
Lahti leachate	53.3 ± 8.1 ^{c}	0.170 ± 0.008 ^{c}	0.314 ± 0.075 ^{ab}	1.84 ± 0.29 ^{d}	5.41 ± 0.28 ^{b}
PE MP	90.0 ± 9.5 ^{a}	0.236 ± 0.009 ^{a}	0.277 ± 0.035 ^{ab}	4.39 ± 0.30 ^{ab}	3.72 ± 0.16 ^{a}
PE leachate	93.3 ± 6.1 ^{a}	0.216 ± 0.008 ^{b}	0.228 ± 0.030 ^{b}	4.33 ± 0.33 ^{b}	4.10 ± 0.23 ^{a}

3.1.1 Total Germination Percentage (G %age)

Normality of variance of the G %age was found to be tenable using a histogram plot; however, homogeneity was not tenable according to Levene's test [$F(8, 45) = 5.256$, $P = 0$]. Kruskal-Wallis H test with pairwise comparisons revealed that there was a significant difference between the means of the groups [$H(8) = 47.85$, $P < 0.001$]. The results showed a severe inhibition in germination for some of the exposure media. Compared to the untreated controls, which had a total germination percentage of 100%, the G %age was 76% lower for new MP and new leachate, 51% lower for Lahti MP, and 47% lower for Lahti leachate ($p < 0.05$). The germination was slightly inhibited (1–10%) also for aged MP, aged leachate, PE MP, and PE leachate, but these differences were not statistically significant ($p > 0.05$). Thus, the new and Lahti exposure media showed considerably higher toxicity than the aged and Port Elizabeth exposure media.

There was no significant difference in total G %age between seeds that had been exposed to MP particles or MP leachates of the same origin (new, aged, Lahti, or Port Elizabeth). Because treatment with MP particles resulted in similar total G %age as treatment with MP leachates, it seems like the particles themselves have little to no effect on the extent of germination. Instead, it is the substances leaching out of the MPs that are responsible for their ecotoxicity. Furthermore, there was no statistically significant difference in total germination percentage between the groups treated with aged MP and aged leachate, compared to PE MP and PE leachate. This indicates that these groups are equally (non-)toxic with respect to inhibition in germination.

For some of the exposure media, especially Lahti MP and Lahti leachate, a considerable amount of seeds was still germinating on day 7. Extension of the germination period with a one or two days would have likely resulted in a somewhat higher total germination percentage for these groups. However, the results clearly show that there

is a significant inhibition in germination of *L. multiflorum* when exposed to microplastic particles and microplastic leachates of new bottle caps and bottle caps collected in Lahti. However, as Joosen *et al.* (2010) explain, "*the total percent germination after a nominated period of time is not very explanatory. It lacks information about start, rate and uniformity of germination, which are essentially parameters of a normally distributed seed population, for many traits such as dormancy, stress tolerance and seed aging*."

3.1.2 Mean Germination Rate (MGR)

Homogeneity and normality of variance for MGR were found to be tenable using Levene's test [$F(8, 45) = 1.494$, $P = 0.186$] and a histogram plot. Normal one-way ANOVA showed that there was a significant difference in means of the MGR between the different exposure media [$F(8, 45) = 45.41$, $P = 0$]. All treatments had lower MGR than the untreated controls, but the difference was not statistically significant for aged MP, aged leachate, or PE MP. The MGR was 29% lower for new leachate, 28% lower for Lahti leachate, 25% lower for new MP, 23% lower for Lahti MP, and 9% lower for PE leachate ($p < 0.05$). This indicates that the germination occurred later and was more spread for seeds exposed to these groups, compared to the controls. There were no significant differences in MGR whether the seeds were exposed to new MP or new leachate, aged MP or aged leachate, or Lahti MP or Lahti leachate. However, seeds exposed to PE leachate had 8% lower MGR than seeds exposed to PE MP ($p < 0.05$), demonstrating a slightly offset and less uniform germination for PE leachate. The MGR for the groups new MP, new leachate, Lahti MP, and Lahti leachate did not significanlty differ from each other ($p > 0.05$), nor did the MGR for the groups aged MP and aged leachate in relation to PE MP and PE leachate.

According to experiments conducted by Soltani *et al*. (2015), MGT is not the mean time to germination; it is merely an index of germination speed and does not tell anything about the time to a specific germination percentage. Since the MGT and MGR deals with the total amount of germinated seeds, not the total seed population, different percentiles of the total population are compared in cases when the final germination percentages differ (Bewly *et al*., 2013; Soltani *et al*., 2015). In this experiment, the total germination percentages varied vastly among the exposure media (24–100%), which may underestimate the differences in MGR when the groups are compared with one another.

3.1.3 Synchronization Index (Z)

Normality of variance for Z was found to be tenable using a histogram plot, but homogeneity was not tenable using Levene's test [$F(8, 45) = 4.224$, $P = 0$]. The non-parametric Kruskal-Wallis H test with pairwise comparison indicated a significant difference in means between the groups [$H(8) = 20.68$, $P = 0.008$]. No group had a statistically significant difference in synchronization index compared to the controls. The only significant difference ($p < 0.05$) in Z in was between the group with the highest value for Z (new leachate) and the three groups with the lowest values for Z (aged MP, aged leachate, and PE leachate). The synchronization index for new leachate was 41% higher than for PE leachate, and 36% higher than for aged MP and aged leachate.

According to these findings, considerably more seeds germinated at the same time in the group treated with new leachate, compared to the group treated with aged MP, aged leachate, or PE leachate. The low Z value for aged MP, aged leachate, and PE leachate implies that the germination in these groups had the least overlap within the time frame. Although generally, groups with higher total G %age seem to have lower values for Z

and vice versa, the controls with 100% total germination and the group new MP with 24% total germination share the value 0.294 for Z. Therefore, it is unwise to draw any conclusions about how Z relates to total G %age in this experiment.

3.1.4 Germination Index (GI)

Normality of variance for the GI values was found to be tenable using a histogram plot, and so was homogeneity according to Levene's test [$F(8, 45) = 1.110$, $P = 0.375$]. Normal one-way ANOVA indicated a significant difference in GI between the groups [$F(8, 45) = 238.1$, $P = 0$]. All treatments showed lower mean GI than the untreated controls in this experiment, but the difference was insignificant ($p > 0.05$) for the groups aged MP, aged leachate, and PE MP. In relation to the controls, the GI was 82% lower for new MP and new leachate, 62% lower for Lahti MP and Lahti leachate, and 11% lower for PE leachate ($p < 0.05$). No statistically significant difference in GI was observed whether the seeds had been exposed to MP particles or the corresponding leachate for any origin of the bottle caps. There was also no significant difference in GI between the aged bottle caps exposure media and the PE bottle caps exposure media ($p > 0.05$). The highest GI was observed for the controls, aged MP, aged leachate, and PE ME exposure media, indicating that the seeds germinated faster and to a larger extent in these groups. New MP and new leachate showed the lowest and slowest germination, followed by Lahti MP and Lahti leachate. The groups' GIs approximately follow the pattern that the higher the G %age and MGR, the higher the GI.

3.1.5 Time to 50% Germination (T_{50})

The variance was not normally distributed for T_{50} according to a histogram of standardized residuals plot, and Levene's test revealed that the variance was non-heterogenous [$F(8, 45) = 3.113$, $P = 0.01$]. The Kruskal-Wallis H test with pairwise

comparisons revealed a significant difference between the group means [H(8) = 42.97, P = 0]. The results showed that T_{50} was 48% higher for seeds treated with new leachate, 47% higher for Lahti leachate, 41% higher for new MP, and 33% higher for Lahti MP compared to the untreated controls ($p < 0.05$). T_{50} was slightly higher also for the other exposure media (1–11%), but these differences were insignificant ($p > 0.05$). No statistically significant difference in T_{50} was found whether the seeds were exposed to MP particles or MP leachate of the same origin, nor if the seeds were treated with exposure media from the aged bottle caps or PE bottle caps. New MP and new leachate were also indistinguishable from Lahti MP and Lahti leachate ($p > 0.05$).

The T_{50} used for these calculations shows the time to 50% germination of the final germination percentage, not the time to 50% germination of the whole population. Treatments with new MP, new leachate, and Lahti MP had a mean total germination percentage of less than 50%, and the lowest observed germination in any of the six replicates was as low as 15% for new MP and new leachate. In order to compare the same germination percentiles of the whole seed population, T_{15} would have been the most appropriate measure. Thus, for new MP and new leachate, who had a mean final germination percentage of 24%, the T_{50} measures the time to 12% germination of the whole seed population. For the controls, on the other hand, the T_{50} measures the time to 50% germination of the whole seed population, since the total germination percentage of the controls were 100%. Because the T_{50} measures widely different percentiles in different populations, it may be somewhat misleading (Bewley *et al.*, 2013). Still, the results clearly show that exposure to microplastic particles and leachates from the new bottle caps and Lahti bottle caps slow down the germination process, since the higher the T_{50} value, the longer the time until half of the germinating seeds have emerged.

In this experiment, higher values for total G %age, MGR, and GI correlate with lower T_{50} values and vice versa. Exposure to new and Lahti MPs and leachates simultaneously results in lower and slower germination, indicating ecotoxicity across multiple germination parameters for these exposure media. Exposure to aged MP, aged leachate, and PE MP did not significanlty affect any of the germination parameters compared to the controls, and treatment with PE leachate displayed a significant difference in germination only with respect to GI. This demonstrates no significant effects on the germination of *L. multiflorum* upon exposure to aged MP, aged leachate, and PE MP, but a slight inhibition in the germination speed when exposed to PE leachate. No unambiguous correlation could be observed between the synchronization index and any of the other germination parameters.

Two recent studies have shown negative effects on the germination of garden cress (*Lepidium sativum*) upon exposure to microplastic particles. Pignattelli *et al.* (2020) found that the total germination was significantly inhibited when exposed to 0.02% (w/w) of several microplastic polymers during acute exposure (6 days) and chronic exposure (21 days). There was also a reduction in germination rate during the chronic exposure experiment for polypropylene and polyethylene MPs. Experiments by Bosker *et al.* (2019) revealed reduced germination of *L. sativum* after 8 h of exposure to MPs of different sizes at a concentration of 107 particles mL^{-1}, but, the effects were no longer detectable after 24 h. Leachates of oxo-degradable polypropylene have furthermore been shown to exert a negative effect on the germination of the plant *Sorghum saccharatum* (Schiavo *et al.*, 2020).

3.2 Growth

As seen in Figure 4, some of the treatments resulted in seedlings with considerably shorter shoots than the controls on day 7 after planting, whereas the effects were less noticeable in other groups. The amount of seeds that resulted in viable seedlings varied vastly among the exposure media, which was addressed in previous sections. The results from the pre-ANOVA statistical analysis on the growth parameters root length, shoot length, root/shoot ratio, and fresh weight, are presented in Table 2 and Figure 5.

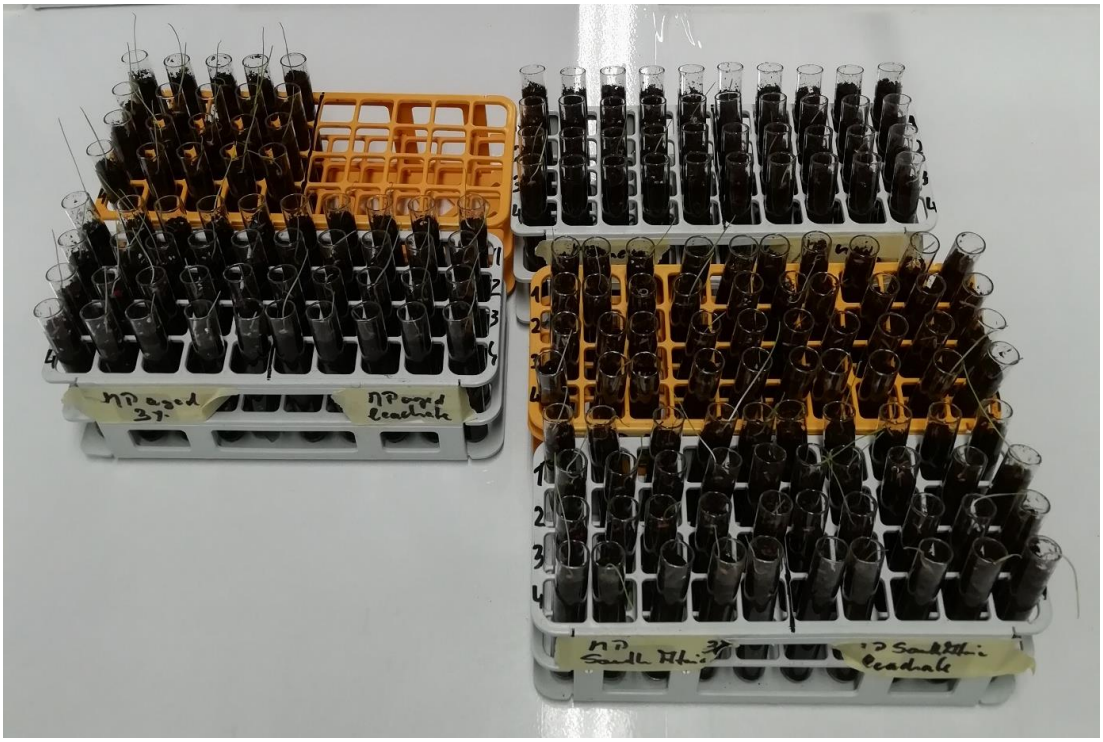

Figure 4. From the upper left corner: seedlings of *L. multiflorum* exposed to control media, new MP, new leachate, aged MP, aged leachate, Lahti MP, Lahti leachate, PE MP, and PE leachate on day 7 after planting the seeds.

Table 3. The results from Levene's test of homogeneity of variance and non-parametric Kruskal-Wallis H test with pairwise comparison for the root lengths, shoot lengths, root/shoot ratio, and fresh weights of the seedlings.

	Levene		Kruskal-Wallis
Variable	df_2	F(8, df_2)	H(8)
Root length	*1071*	18.75 ***	678.7 ***
Shoot length	*1071*	18.75 ***	743.4 ***
Root/shoot ratio	*751*	7.576 ***	52.64 ***
Fresh weight	*1071*	17.06 ***	641.6 ***

* p < 0.05, ** p < 0.01, *** p < 0.001

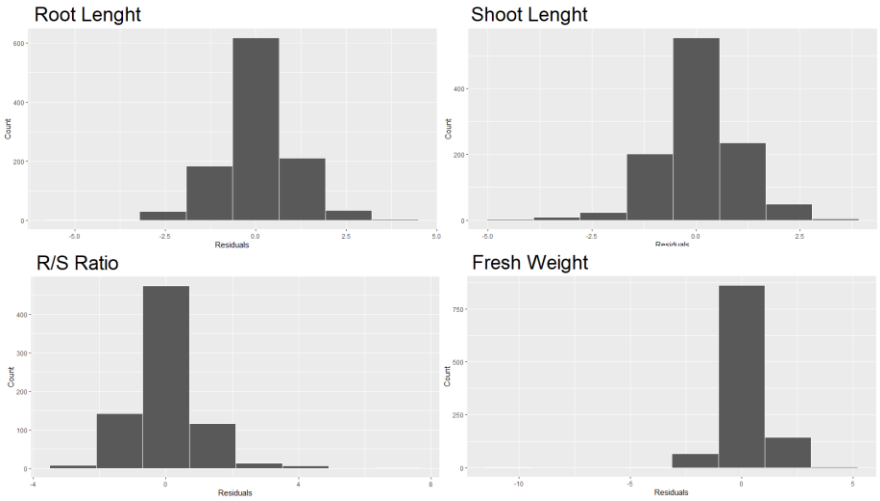

Figure 5. Histogram plots of residuals showing the normality of variance for the root lengths, shoot lengths, root/shoot (R/S) ratio, and fresh weights.

3.2.1 Root and Shoot Length

Normality of variance was found to be tenable using histogram plots; however, homogeneity was not tenable according to Levene's test [root length: F(8, 1071) = 18.75, P = 0; shoot length: F(8, 1071) = 18.75, P = 0]. The non-parametric Kruskal-Wallis H test with pairwise comparison showed significant differences in means between the groups [root length: H(8) = 678.7, P = 0; shoot length: H(8) = 743.4, P = 0]. The root and shoot lengths for the different exposure media, expressed in means ± standard deviation, are presented in Figure 6. Seedlings of *L. multiflorum* showed significant reduction in root length on day 7 for all exposure media except PE MP, in

relation to the untreated controls ($p < 0.05$). Most severely affected were seedlings exposed to new MP and new leachate, whose root lengths were 84% and 91% shorter than the controls. The reduction in root length was 73% for Lahti MP and 67% for Lahti leachate. Seedlings from the groups aged MP and aged leachate showed less but still significant inhibition in root growth; on average 15% for aged MP and 24% for aged leachate. PE leachate was found to have the smallest, still statistically significant reduction in root length; on average 13% shorter roots than the controls on day 7 after planting. For PE MP, the root length was on average 10% shorter than the controls, but this difference was statistically insignificant ($p > 0.05$). Soil containing 3% w/w MP particles from bottle caps collected in Port Elizabeth was thus the only exposure media included in this experiment that did not significanlty reduce the root length of *L. multiflorum*.

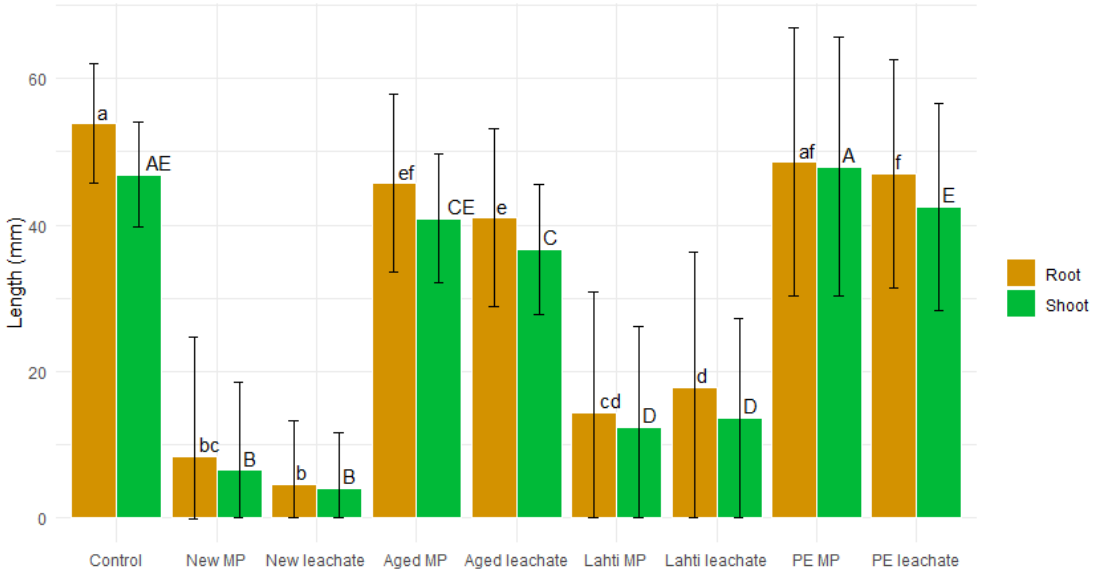

Figure 6. The root and shoot lengths of seedlings exposed to different treatments, expressed in means ± standard deviation. Bars of the same colour not sharing the same letters differ significantly at the 0.05 level of probability.

There was no statistically significant difference in root growth whether the seedlings had been exposed to MP particles or MP leachate of the same origin for any of the

groups. The root lengths were on average slightly shorter for seedlings treated with new leachate, aged leachate, and PE leachate compared to new MP, aged MP, and PE MP, but these differences were insignificant ($p > 0.05$). Concerning the bottle caps collected in Lahti, exposure to Lahti MP resulted in somewhat shorter roots than for seeds exposed to Lahti leachate, but this difference was also statistically insignificant. Furthermore, no significant distinction could be made between the root lengths of seedlings exposed to new MP and Lahti MP, nor between seedlings exposed to aged MP and PE MP or PE leachate. Between all other pairs of groups, the difference in root length was significant ($p < 0.05$).

The shoot growth was significantly inhibited on day 7 for seedlings treated with all exposure media except PE MP and PE leachate, compared to the controls ($p < 0.05$). Like the root lengths, the shoot lengths were most severely reduced for the exposure media from new bottle caps, followed by Lahti bottle caps and aged bottle caps. Seedlings exposed to new MP and new leachate had an average reduction in shoot length of 86% and 91%. For the groups Lahti MP and Lahti leachate, the shoot length was reduced with 74% and 71%. Exposure to aged MP and aged leachate resulted in 13% and 22% shorter roots. The shoot length of seedlings exposed to PE MP was on average 2% longer and exposed to PE leachate 9% shorter than the controls, but neither of these findings were statistically significant ($p > 0.05$). No statistically significant difference in shoot length between seedlings treated with microplastic particles and microplastic leachate of the same origin could be observed for new bottle caps, aged bottle caps, or Lahti bottle caps. There was, however, an 11% reduction ($p < 0.05$) in shoot length for seedlings exposed to PE leachate in relation to PE MP. The pairwise comparison showed significant differences between all other pairs of groups, except for between aged MP and PE leachate exposure media.

According to these findings, microplastic particles and microplastic leachate can have serious effects on the root and shoot growth of terrestrial plants. This is in accordance with previous studies. Boots *et al.* (2019) found that the shoot length of *Lolium perenne* was inhibited by 19% compared to the controls when exposed to polyactic acid (PLA) MPs, and reduction in the shoot length of *L. sativum* was also observed by Pignattelli *et al.* (2020) upon exposure to MPs of several polymer types. Reduced root lengths were also demonstrated for the plant *Vica faba* when exposed to 5 µm polystyrene MPs at concentrations of 50 and 100 mg/L (Jiang *et al.*, 2019). In another study by Bosker *et al.* (2019), significant differences in root growth of *L. sativum* were observed after 24 h, but not after 48 or 72 h of exposure to MP particles. The effects on the root lengths were both positive and negative depending on the particle size, and there was little to no effect on the shoot growth. Luo *et al.* (2019) further found that leachate from 1.6 g L^{-1} polyurethane foam (PUF) MPs inhibited the growth of the algae *Chlorella vulgaris* by about 10.3% compared to the control after 5 days of incubation.

In the present study, the highest reduction in both root and shoot growth was observed for seedlings of *L. multiflorum* treated with new leachate and new MP, followed by a considerable reduction for Lahti MP and Lahti leachate, and a moderate reduction for aged leachate and aged MP. Seedlings treated with PE leachate had a slight reduction in root length but not in shoot length, whereas treatment with PE MP did not significantly affect the root or shoot lengths at all. The difference between new and aged microplastics indicates that ageing drastically decreases the ecotoxicity of MPs. This is possibly because a large part of the hazardous additives has already leached out of the aged material over time, and therefore less toxins are present in the aged MPs than in the new. The low ecotoxicity of PE microplastics compared to Lahti microplastics could be explained by the warmer climate in South Africa, which

contribute to quicker degradation of the toxic components than in the colder environment of Lahti. Another reason could be that the bottle caps collected in Port Elizabeth are older than the ones from Lahti. In this experiment, the root and shoot growth inhibition was similar regardless of whether the seeds had been exposed to particles or leachates of MPs of the same origin. This suggest that it is the chemicals leaching out of the plastic material, not the particles themselves, that are responsible for ecotoxicological effects of microplastics on *L. multiflorum* seedlings.

The mechanisms through which microplastics affect plant growth are not known, but Rilling *et al.* (2019) propose a few suggestions. Microplastic present in the soil changes the soil structure, potentially decreasing penetration resistance for roots and increasing aeration, which could lead to enhanced root growth. On the other hand, the addition of MPs to soil may create channels for water movement that increase water evaporation, which poses a risk of the soil drying and thereby decreased plant growth. Changes in soil structure may also affect the microbial community composition, which indirectly have consequences for plant growth. Plastic particles may also act as vectors for pollutants already present in the soil, transferring these to plants and affecting their growth. Pollutants already present in the plastic material are also likely to interfere with the growth. However, the adsorption of pollutants onto microplastic particles may also decrease the bioavailability of the pollutants, and thereby protect the plants.

Rillig *et al.* (2019) further suggest that on a longer time scale, microbial nutrient immobilization will likely occur when the carbon-rich, low-in-nitrogen plastic material is slowly degraded. Microplastics in the soil are also likely to further break down into nanosized particles, which may be directly taken up by plant roots and potentially damaging them. This could lead to the nanoplastics entering the food chain. On the other hand, nanoplastics might strongly sorb to the soil surface, decreasing the

potentially toxic effects on plant growth. Since the mechanisms by which MPs affect plant growth are largely unknown and seem to simultaneously be able to cause both inhibitory and promoting effects, it is of great importance to further investigate this topic.

3.2.2 Root/Shoot (R/S) Ratio

Normality of variance of the R/S ratio was found to be tenable using a histogram plot, but homogeneity was not plausible according to Levenes's test [$F(8, 751) = 7.576$, $P = 0$]. Kruskal-Wallis H test with pairwise comparisons demonstrated significant differences in means between the groups [$H(8) = 52.64$, $P = 0$]. Seedlings treated with Lahti leachate had the highest R/S ratio of all the exposure media included in this experiment, as shown in Figure 7. Lahti leachate had a 18% higher R/S ratio than the controls ($p < 0.05$) and was the only group that significantly differed from the controls. Furthermore, the R/S ratio of seedlings exposed to Lahti leachate was 21–34% higher ($p < 0.05$) than for the groups treated with aged MP, aged leachate, PE MP, and PE leachate.

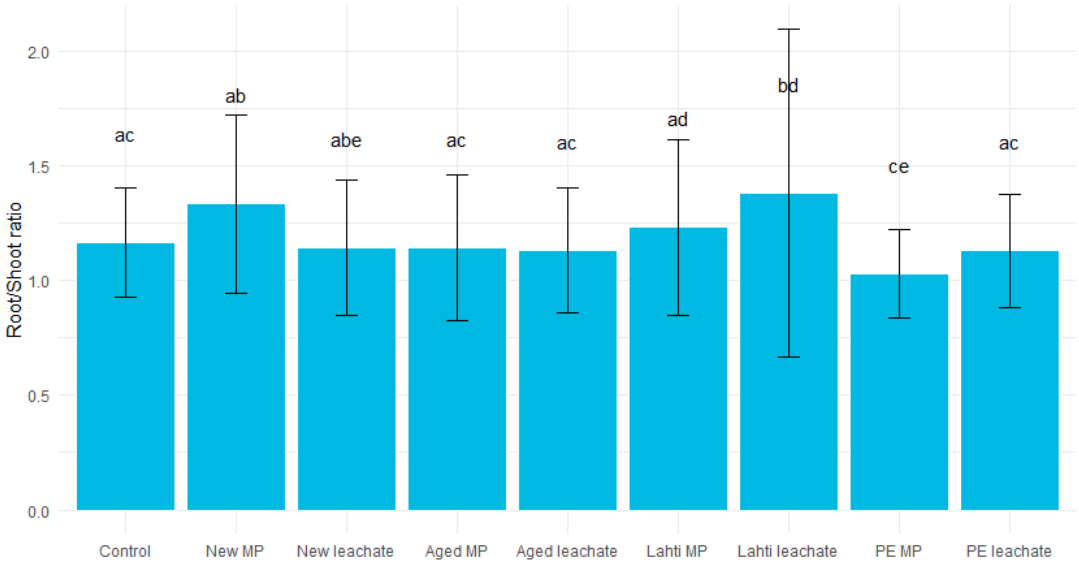

Figure 7. The root/shoot ratio of seedlings exposed to different treatments, expressed in means ± standard deviation. Bars not sharing the same letters differ significantly at the 0.05 level of probability.

The lowest R/S ratio was seen for seedlings treated with PE MP. These had a 12 % lower mean R/S ratio than the controls, but difference was not statistically significant. However, in relation to new MP, Lahti MP, and Lahti leachate, the R/S ratio for PE MP was 17–25% lower ($p < 0.05$). No significant difference in R/S ratio was observed whether the seedlings had been exposed to MP particles or MP leachate of bottle caps of the same origin. According to these findings, seedlings of *L. multiflorum* display a stress reaction when treated with the Lahti leachate exposure media. This indicates that the chemicals leaching out of the Lahti MPs have ecotoxic effects on the plant. Since treatment with Lahti MPs did not result in any significant effect to the R/S ratio, it is possible that the addition of MP particles cancels out the negative effect of the leachate. In a study by Boots *et al.* (2019), *L. perenne* planted in soil containing 0.1% w/w HDPE MP resulted in seedlings with a 35% higher root/shoot ratio than the controls, demonstrating a stress response in the plant upon exposure to MPs. Treatment with synthetic fibers (acrylic and nylon mixture) and biodegradable polyactic acid (PLA) did not significantly affect the R/S ratio of *L. perenne* in the study, which indicates that the ecotoxicity of MPs vary depending on the polymer type. A possible explanation for this could be that different materials have different compositions of plastic additives.

3.2.3 Fresh Weight

Normality of variance of the fresh weight was found to be tenable, but homogeneity was not according to Levene's test [$F(8, 1071) = 17.06$, $P = 0$]. Kruskal-Wallis' non-parametric H test indicated significant differences in means between the groups [$H(8) = 641.6$, $P = 0$]. In Figure 8, the fresh weights of the seedlings are presented as means ± standard deviation together with the results from the ANOVA. Seedlings exposed to all microplastic particles and leachates included in this experiment had significantly ($p < 0.05$) lower fresh weight than the untreated controls on day 7. As for root and shoot

length, most extreme reductions in fresh weight were seen for the new MP and new leachate exposure media. These groups had on average 84% and 82% lower fresh weights than the controls. Treatment with Lahti MP and Lahti leachate also resulted in severe reductions in seedling fresh weight; on average 77% and 79%. The reduction in fresh weight for seedlings exposed to aged MP and aged leachate were 23% and 39%, and for PE MP and PE leachate the fresh weights were 31% and 43% lower than the controls.

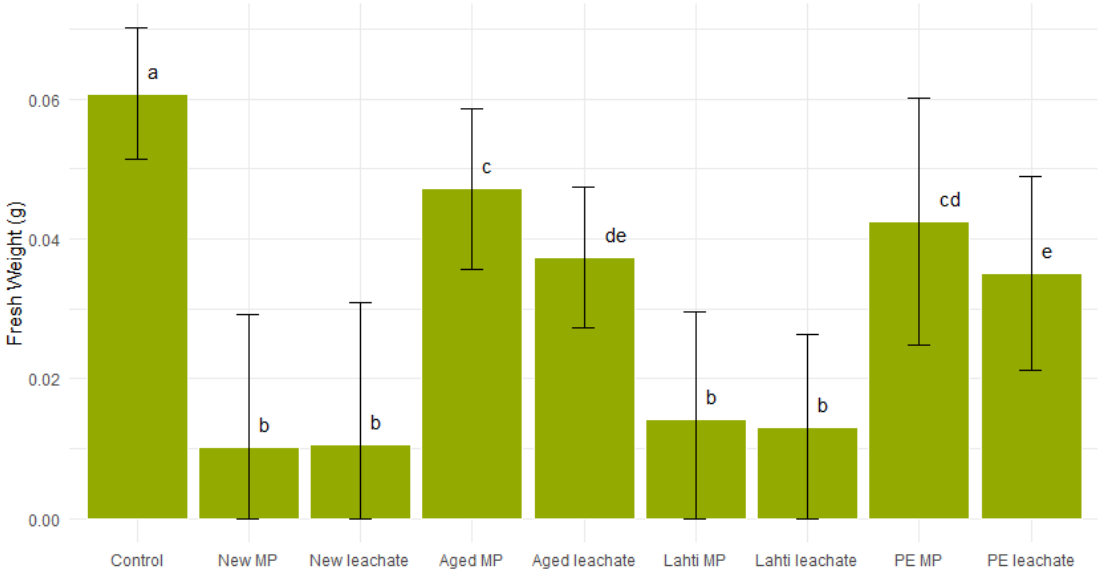

Figure 8. The fresh weight of seedlings exposed to different treatments, expressed in means ± standard deviation. Bars not sharing the same letters differ significantly at the 0.05 level of probability.

There was no significant difference in fresh weight between seedlings exposed to new MP and new leachate, nor between Lahti MP and Lahti leachate. However, the fresh weight for MP particles differed from the corresponding MP leachates for the aged bottle caps and Port Elizabeth bottle caps. The fresh weight for aged leachate was on average 21% lower than for aged MP, and PE leachate was on average 17% lower than for PE MP ($p < 0.05$). This indicates that the leachates from aged and Porth Elizabeth MPs had higher ecotoxicology than the microplastic particles. A possible explanation

for this could be that the particles had some positive effect on the soil composition and thereby the growth, such as more aeration, which the groups treated with only leachate lacked. However, this effect was not seen for the more toxic new and Lahti material, possibly because the toxicity of the leached substances overrode this effect. The differences in fresh weight between the groups new MP, new leachate, Lahti MP, and Lahti leachate were not statistically significant, nor was the difference between aged leachate and PE MP, aged leachate and PE leachate, and aged MP and PE MP. However, seedlings exposed to PE leachate had 26% lower fresh weight than seedlings exposed aged MP ($p < 0.05$), again suggesting that MP leachates by themselves may be more toxic than when accompanied by MP particles.

The reduction in fresh weight for new MP and new leachate (82–84%) follow approximately the same pattern as the reduction in root (84–91%) and shoot (86–91%) length. The same applies to Lahti MP and Lahti leachate, whose fresh weights were 77–79% lower than the controls, compared to 67–73% shorter roots and 71–74% shorter shoots than the controls. However, the reduction in fresh weight for aged MP, aged leachate, PE MP, and PE leachate were considerably higher than the reduction in root and shoot lengths. The seedlings exposed to these media were thus only slightly shorter, but significantly thinner than the controls. This effect was particularly distinctive for the Port Elizabeth exposure media. Hence, although there was no or only a slight reduction in seedling length for *L. multiflorum* exposed to leachates compared to the corresponding MP particles for aged or Porth Elizabeth exposure media, ecotoxicological effects could be observed as a reduction in fresh weight. It is also worth noting, that while exposure to the aged and Port Elizabet media did not significantly decrease the total germination percentage of *L. multiflorum*, the plant biomass of the seedlings was significantly reduced also in these groups.

Previous experiments measuring plant weights after exposure to MPs have demonstrated varying effects. In a study on wheat (*Triticum aestivum*) by Qi *et al.* (2018), the shoot biomass was lower than the controls ($p < 0.05$) when exposed to starch-based biodegradable MPs, but not when exposed to LDPE MPs, after a period of 14–40 days. The biodegradable MPs had negative effects on the shoot biomass also after 2 months harvest. Both plastic materials resulted in lower root biomass at 2 months harvest, but none of them exhibited lower root or shoot biomass after 4 months. Pignattelli *et al.* (2020) found that the shoot biomass of *L. sativum* was reduced upon exposure to polyethylene MPs during acute exposure (6 days), and the shoot biomass was negatively affected when exposed to polyethylene and polypropylene MPs during chronic exposure (21 days). In a study by Boots *et al.* (2019), exposure to MPs resulted in higher root biomass of *L. perenne*, and in another study, Jiang *et al.* (2019) found that exposure to 5 mm polystyrene MP in concentrations of 10, 50, and 100 mg/L resulted in significantly lower fresh weight of *Vicia faba* roots compared to the control ($p < 0.05$).

4 Conclusions

In line with the hypothesis, this study showed that microplastic particles and microplastic leachates may severely inhibit the germination and growth of the terrestrial plant *L. multiflorum*. New MPs showed a significanlty higher toxicity profile than artificially aged MPs, which indicates that the negative effects on the plant's germination and growth decreases with the age of the MPs. A reason why aged MPs may be less toxic than new ones could be that much of the toxic additives have already leached out of the aged plastic material over time. MPs from the Lahti region were considerably more toxic than MPs from Port Elizabeth, implying that regional

environmental factors have an impact on the toxicity of MPs. A possible explanation could be that the warmer climate of South Africa leads to a faster breakdown of hazardous substances in the plastic than the cooler temperatures in Lahti. The MP leachates gave rise to equal or slightly higher inhibition in germination and growth as the MP particles, implying that it is the substances leaching out of the MPs that are responsible for the adverse effects.

In this experiment, pure garden soil was used as the growth medium. In nature, the soil may be polluted with other chemicals which may increase the negative effects of MPs by sorption of these chemicals onto the MPs. The current experiment was conducted under laboratory conditions with one single species, and the concentrations of MPs and leachates used were relatively high. Thus, this experiment does not directly correspond to conditions in nature, but high concentrations of MPs may nevertheless occur in some environments and impair the germination and growth of terrestrial plants. As the plastic pollution is not expected to go away anytime soon, it is important to further examine how MP particles and leachates affect different organisms. Future research could investigate, for example, different plant species, different polymer types, and different concentrations of MPs than the ones used in the present study.

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